The Role of Gender in Determining the Duration of Topical Mosquito Repellent Efficacy.

Andrea Drago[1], Mara Moreno[2], Reda Ibrahm[3].

[1]Entostudio Srl, Ponte San Nicolò, Italy

[2]Henkel Ibérica S.A, Research and Development (R&D) Insect Control Department, Barcelona, Spain,

[3]Entomology Department, Kafrelsheikh University, Egypt.

emca European Mosquito Control Association
XI International Conference
7-10 November 2023, Palma, Spain
SHAPING THE FUTURE OF VECTOR CONTROL IN EUROPE

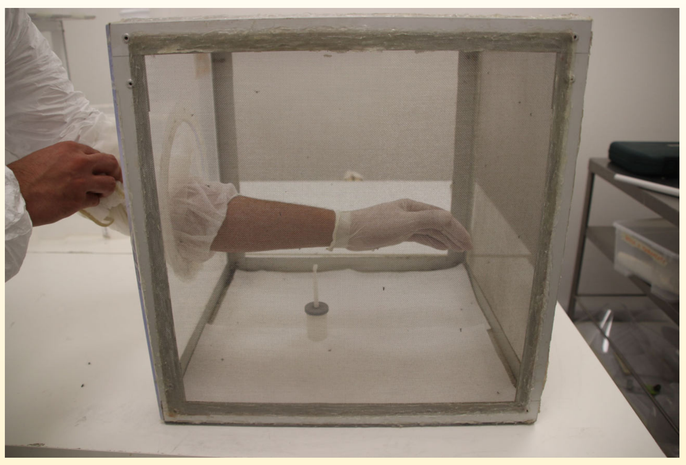
THE ARM IN CAGE TEST

The assessment of the effectiveness of topical repellents is typically carried out using a procedure known as the "arm-in-cage" test. This method involves introducing, at specific time intervals, a treated arm into an enclosure where hungry mosquitoes have been released. Several factors within the test can be adjusted, including the mosquito species, mosquito strain, cage size, mosquito population size, mosquito age, timing of observations, and the frequency of these observations. All of these variables have the potential to impact the level of mosquito biting and the overall effectiveness of the repellents, as noted by Donald et al. in 1998.

THE CALCULATION OF FULL PROTECTION DURATION - THE COMPLETE PROTECTION TIME

The approach used to assess the effectiveness of repellents can significantly impact the outcome in terms of protection. The complete protection time (CPT) is determined in various ways depending on the guidelines in use. The World Health Organization (WHO) defines CPT as the duration, in minutes, that transpires from the moment the repellent is applied until the first instance of a mosquito landing and/or probing. Consequently, if the initial mosquito bite occurs after 4 hours, the CPT is recorded as 4 hours.

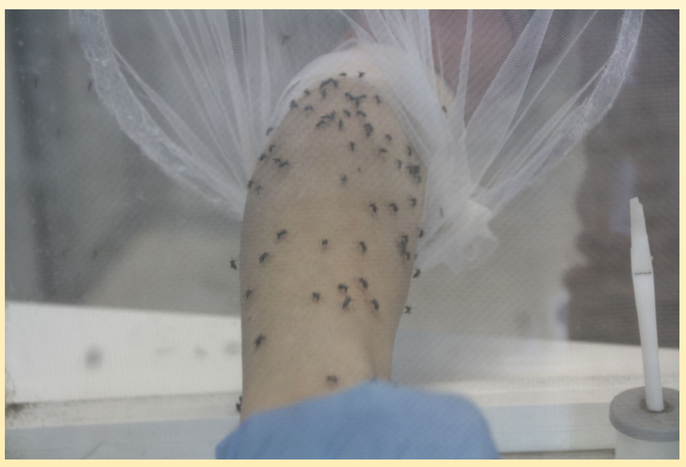
The Environmental Protection Agency (EPA) also employs the WHO's definition of CPT but with a nuanced variation. In the EPA's framework, efficacy failure is not solely determined by the first bite; it encompasses confirmed mosquito bites. Until the release of version 3.0 of the European Chemicals Agency (ECHA) guidelines, CPT was aligned with EPA's criteria. However, in subsequent versions such as 4.0, 4.1, and 5.0, the CPT has been shortened by 1 hour (or 30 minutes).



GENDER'S INFLUENCE ON CPT

Numerous guidelines, including the ECHA Guidance on the BPR: Volume II Parts B+C Version 5.0 (November 2022), EPA OPPTS 810.3700: Insect Repellents for Application to Human Skin, and WHO/HTM/NTD/WHOPES/2009.4 Guidelines for Efficacy Testing of Mosquito Repellents for Human Skin, recommend the inclusion of volunteers from both genders in repellent efficacy testing. However, the fundamental question arises: Is there truly a disparity in attractiveness between males and females, and does one's gender genuinely impact the effectiveness of topical repellents?

Several studies have explored this significant matter, typically concentrating on a single active ingredient or a specific mosquito species, yielding conflicting findings. The role of gender in efficacy evaluations has a bearing on participant selection and the trustworthiness of the outcomes. Yet, due to the limited information available, it remains challenging to definitively determine whether a tangible divergence in attractiveness exists between genders. Furthermore, the question of whether this disparity in attractiveness correlates with the efficacy of topical repellents remains unanswered.



PRODUCT	GROUP	formulation	active ingredient	content of AI %	dosage mg/cm2	duration assesment min	n mosquitoes or ticks/hour	species	sex	CPT
2	A	aerosol	icaridin	12%	1,66	3	50	Aa	m	8
		aerosol	icaridin	12%	1,66	3	50	Aa	f	4
		aerosol	icaridin	12%	1,66	3	50	Aa	m	8
		aerosol	icaridin	12%	1,66	3	50	Aa	m	8
		aerosol	icaridin	12%	1,66	3	50	Aa	f	8
		aerosol	icaridin	12%	1,66	3	50	Aa	f	7
		aerosol	icaridin	12%	1,66	3	50	Aa	m	8
		aerosol	icaridin	12%	1,66	3	50	Aa	f	5
		aerosol	icaridin	12%	1,66	3	50	Aa	m	6
		aerosol	icaridin	12%	1,66	3	50	Aa	m	4
	B	aerosol	icaridin	12%	1,66	3	100	Cp	f	8
		aerosol	icaridin	12%	1,66	3	100	Cp	m	8
		aerosol	icaridin	12%	1,66	3	100	Cp	f	2
		aerosol	icaridin	12%	1,66	3	100	Cp	f	8
		aerosol	icaridin	12%	1,66	3	100	Cp	f	6
		aerosol	icaridin	12%	1,66	3	100	Cp	m	4
		aerosol	icaridin	12%	1,66	3	100	Cp	m	5
		aerosol	icaridin	12%	1,66	3	100	Cp	m	6
		aerosol	icaridin	12%	1,66	3	100	Cp	f	6
		aerosol	icaridin	12%	1,66	3	100	Cp	m	5

Fig 1: example of the analyzed data.

MATERIALS AND METHOD

The data utilized in this analysis were collected from efficacy tests conducted on various commercial topical repellents over the course of several years. These tests followed the "Arm in Cage" method, as outlined in ECHA guidelines version 3.0. However, it's important to note that numerous parameters were not held constant during these assessments. Several factors were adjusted depending on the specific product and its commercial claims. These factors included the species used (such as mosquitoes like *Ae albopictus, Ae aegypti, An gambiae, Cx pipiens, Cx quinquefasciatus,* and ticks like *Ix ricinus* nymphs), the active ingredient (AI) employed (which could be Icaridin, Citrepel, IR3535, PMD, Citrodiol, N,N-diethyl-m-toluamide-DEET), the concentration of the active ingredient, the formulation type (e.g., lotion, aerosol, wipe, roll-on, gel), and the dosage. Additionally, certain parameters were adjusted to accommodate specific requests from the parties conducting the tests, such as the duration of the assessments (ranging from 3 to 5 minutes) and the number of mosquitoes involved..

Group/Compound	P value	Significance		Group/Compound	P value	Significance
1A	0.262			21A	0.239	Not significant
1B	0.367			22A	0.859	Not significant
1C	0.740			23A	0.308	Not significant
1D	0.784	Not significant		24A	0.468	Not significant
2A	0.397	Not significant		24B	------	No difference as all CPT = 0
2B	0.764			24C	0.937	Not significant
3A	0.399	Not significant		25A	0.401	Not significant
4A	0.764	Not significant		25B	0.346	Not significant
5A	------	No difference as all CPT = 8		26A	0.495	Not significant
5B	------	No difference as all CPT = 8		26B	0.724	Not significant
5C	0.025	Significant F > M		27A	0.638	Not significant
6A	0.887	Not significant		27B	0.789	Not significant
7A	1.000	Not significant		28A	0.219	Not significant
7B	0.414	Not significant		28B	------	No difference as all CPT = 0
8A	------	No difference as all CPT = 8		29A	------	No difference as all CPT = 0
8B	0.116	Not significant		29B	------	No difference as all CPT = 0
9A	0.785	Not significant		30A	0.180	Not significant
10A	0.141	Not significant		31A	------	No difference as all CPT = 0
11A	0.658	Not significant		32A	0.339	Not significant
12A	0.677	Not significant		33A	0.242	Not significant
12B	0.819	Not significant		33B	0.421	Not significant
12C	0.073	Not significant		33C	0.471	Not significant
13A	0.272	Not significant		33D	0.809	Not significant
14A	0.923	Not significant		34A	0.099	Not significant
15A	0.219	Not significant		34B	0.526	Not significant
16A	0.789	Not significant		35A	0.861	Not significant
17A	0.789	Not significant		36A	0.100	Not significant
18A	------	No difference as all CPT = 1		37A	0.172	Not significant
19A	0.537	Not significant		38A	0.347	Not significant
20A	0.402	Not significant				

Fig.2 : Non significancy of the gender on CPT

RESULTS

Data analysis was conducted employing Minitab® software version 17. A one-way analysis of variance (ANOVA) was employed to assess the impact of gender on the complete protection time (CPT).

Regarding the "T-Test," it was used to compare multiple replicates carried out by various volunteers, all of whom adhered to the same parameters encompassing product, dosage, assessment duration, assessment frequency, mosquito species, and mosquito count. The effect of gender on the CPT was quantified by evaluating 414 repetitions across 38 different products.

A total of 59 analyses were conducted, and it's noteworthy that **only one instance revealed a discernible difference between male and female volunteers.**

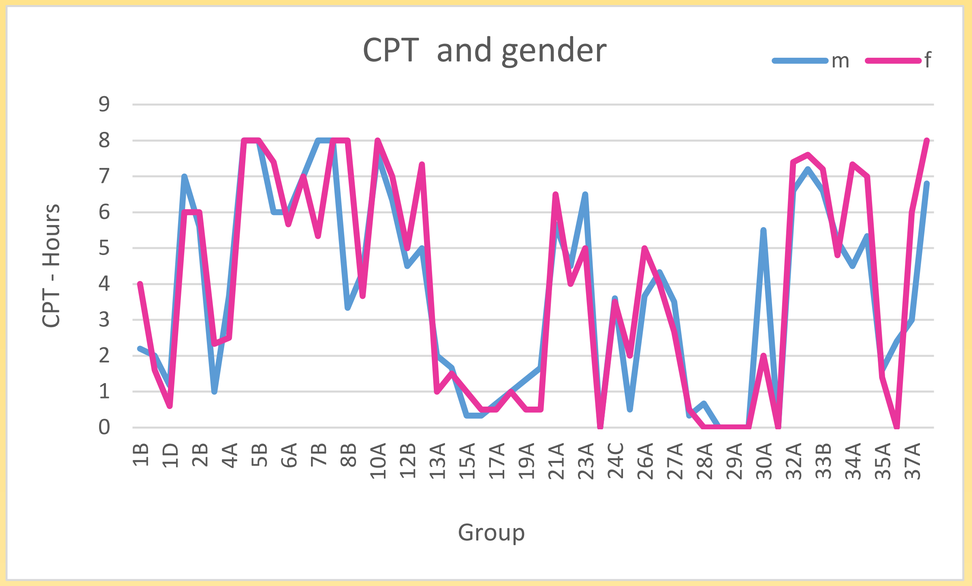


Fig. 3: ***Analysis of difference between males and females in terms of duration of CPT: lines blue (for males) and pink for females) indicate the CPT for each group.***